REMARKS

Claim 7 has been amended herein. Claims 1-21 remain in the case. Favorable reconsideration is respectfully requested.

The following remarks address the issues presented in the Office Action in the order of their appearance:

The Restriction Requirement:

Applicants continue their traversal of the original restriction requirement as being clearly improper. Applicants further traverse the reformulated restriction requirement and the making of the reformulated requirement final as being unquestionably improper.

Regarding the reformulated restriction requirement, Applicants responded to the earlier restriction based upon the following grouping of Claims:

Group I: Claims 1-5 and 7-21; and

Group II: Claim 6.

In the present Office Action, the Office unilaterally regrouped the claims as follows:

Group I: Claims 1-5 and 7-12; and

Group II: Claims 6 and 12-21.

The Office then made the new restriction requirement final. The finality of the restriction requirement as amended is traversed because the Office is not at liberty to make the new restriction requirement final. Applicants have not been given an opportunity to respond to the new restriction requirement on the merits. The Office simply has no basis upon which to make a new restriction requirement, and then to make the new restriction a "first Office Action" final. Applicants are entitled to respond to the new restriction requirement on the merits. Thus, Applicants request that the finality of the restriction requirement be withdrawn.

Applicants also traverse the new restriction requirement because the Office has failed entirely to provide any reasons or examples to support the new restriction, as required by MPEP §803. Applicants therefore submit that the new restriction requirement is improper on its face.

Regarding the prior restriction requirement, Applicants note that restriction is to be based upon the invention "as claimed." See MPEP §803.

Thus, for example, the Office makes an improper argument in stating that the "end point" of Claim 4 is an inclusion body, while the "end point" of Claim 6 is a "soluble active streptokinase." The distinction is meaningless because the invention as claimed is, in both of Claims 4 and 6, is to a method of producing streptokinase.

Regarding the word "another" as used in Claim 6, the present wording of Claim 6 does not explicitly or implicitly indicate that an "alternative" expression construct is used. Rather, "another" as used in the Claim 6 indicates that a second construct is used, in addition to the construct recited in Claim 1. This point is clearly expressed in the specification at page 3, last paragraph. Applicants respectfully reply that the claims are to be read in light of the specification, and not in a vacuum.

Lastly, Applicants are not taking any position on the merits with regard to the patentable distinctness of the claims. Applicants are, however, pointing out for the record that the Office has a statutorily defined burden it must carry to establish a proper case for restriction. That burden was not met in the original restriction requirement, nor has that burden been met in the comments advanced by the Office in the Action dated May 20, 2004. The Office has made no attempt at all to support the new restriction requirement present in the present Office Action. Thus, the reformulated restriction requirement is improper on its face. Applicants thus continue their traversal of the restriction requirement, both as originally promulgated and as reformulated in the present Office Action.

Withdrawal of the restriction requirement is respectfully requested.

Rejection of Claims 1, 2, 4, 5, 7-13, and 15-21 Under §112, First Paragraph (Written Description):

This rejection is respectfully traversed because the two cases cited in support of this rejection, *The Regents of the University of California v. Eli Lilly & Co.* and *Fiers v. Revel*, 25 USPQ2d 1601, **are not** controlling law. First, the discussion of the written description requirement from the *Fiers* case is contained in *dictum* and **was not** the holding of the case. Therefore, it is improper for the Office to cite *Fiers* as being the controlling law on the issue of written description. Second, the quoted language from the *Lilly* case is specific to a set of facts that **are not** analogous to the present application and cannot logically be extended to the present application.

In short, the citations to *Fiers* case and the *Lilly* case in support of this rejection are inapposite. Specifically, the quote from *Fiers* that:

> Disclosure sufficient to satisfy the written description requirement... must include a precise definition of DNA, such as by structure, formula, chemical name, **or** physical properties (emphasis added),

is contained in **dictum** and **was not** the holding of the court. Consequently, the above quote from *Fiers* is not a controlling statement on the written description requirement. (Note also that the string of requirements is in the alternative.) Contrary to Judge Lourie's above-quoted dictum from *Fiers*, it has **never** been the law that an Applicant must know the structure of a compound (DNA, protein, or otherwise) in order to satisfy the requirements of §112, first paragraph (written description).

The controlling law in this instance is provided by *In re Fisher*, 166 USPQ 18 (CCPA 1970). In *Fisher*, the question was whether the claims of a CIP application (drawn to a protein) were entitled to the filing date of the parent application. In *Fisher*, it was undisputed that the amino acid sequence of the protein claimed in the CIP was not disclosed in the parent application, and further that *Fisher* did not know the amino acid sequence at the time the parent application was filed (166 USPQ at 21). The only

disclosure in the parent application was a process for extracting the protein from the pituitary glands of certain animals. However, an article which appeared **after** the filing of the CIP application confirmed that the claimed sequence was, in fact, the sequence of protein described in the parent application. The CCPA **held** that the claimed structure was inherent in the description contained in the parent application, and therefore the parent application described the protein to the level required by §112, first paragraph.

As applied to the present situation, *Fisher* makes clear that the written description requirement of §112 is satisfied by the disclosure of a biological product having specific and **known biological properties** - in this instance, the ability to catalyze a specific reaction (*i.e.*, thrombolysis). The present specification clearly describes (by way of nucleotide sequence) a specific streptokinase. In short, the law does not mandate that the specification recite every single nucleic acid sequence which encodes a streptokinase in order to satisfy §112, first paragraph (written description).

The Regents of University of California v. Eli Lilly & Co. is also inapposite to the present case because in *Regents*, the plaintiffs **never** described **any** aspect of the claimed human cDNA beyond the amino acid sequence it was to encode. The only cDNA sequence contained in the disputed specification in *Regents* was that of a rat cDNA analogous to the claimed human cDNA. Having failed to describe **any** characteristics of the claimed **human** cDNA itself (*e.g.*, structure, formula, or physical properties, as noted in *Fiers*), the Court in *Regents* held that plaintiffs did not adequately describe a cell transformed to contain a **human** cDNA.

These facts simply do not apply to the present claims. Applicants have quite clearly described a streptokinase, its structure, and its function. As described in the paragraphs that follow, the term "streptokinase" is an art-recognized term that clearly conveys to one of skill in the art the parameters of the claimed invention.

As articulated by the Office, this rejection boils down to the art-recognized definition of the term "streptokinase." The Office has taken the position that

"streptokinase" is not a sufficiently precise word to convey to one of skill in the art that the present Applicants were in possession of the invention as broadly as it is currently claimed. In other words, that the term "streptokinase" is not a sufficiently precise noun to describe the genus of enzymes that function as "streptokinases." Applicants respectfully traverse this rejection because, by extremely long-standing tradition, enzymes have and continue to be be named, described, cataloged, listed, compiled, and indexed by functional names ending in -ase. The entire Enzyme Classification system, the means by which enzyme identity is cataloged worldwide, is predicated upon functional definitions.

By way of objective evidence, attached hereto as Exhibit A is a brief history of the institution of the Enzyme Classification system. The history attached as Exhibit A was produced by the International Union of Biochemistry and Molecular Biology (IUBMB), in conjunction with the International Union of Pure and Applied Chemistry, the international body that governs the systematic nomenclature of enzymes and other chemicals. As noted on the second page of Exhibit A, the present "EC" system was first promulgated in 1961, and has been amended five times since, as new enzymes have been discovered.

Streptokinases fall within EC 3.4.21.7, namely hydrolases, that act on peptide bonds, that are serine endopeptidases, and that act upon a plasmin substrate. In short, the phrase "streptokinase" is very, very precise. See Exhibits B and C, which are excerpts from the IUBMB web page and the Brookhaven Protein Data Base, respectively, for this EC.

See also the discussion in the present specification at page 1, last paragraph, to page 3, second paragraph. This passage references several different streptokinases known in the art, and two streptokinases that can be purchased commercially (*e.g.*, U.S. Patent No. 3,885,065, German patent publication IPC C12 N15/00, ATCC 12449, and ATCC 9542). The present application also explicitly cites U.S. Patent No. 5,296,366, which

describes the transgenic production of streptokinase using the *trp* promoter in *E. coli*. In short, the present specification contains an ample description of the structure and function of several enzymes that are "streptokinases." Thus, the Office is not correct in noting that the application discloses but a single type of streptokinase.

Note also that streptokinases, as a genus of enzymes, not only have the same functionality, they are also remarkably conserved. For example, the streptokinase C precursor, the streptokinase G precursor, streptokinase SKC-2 equisimilis, and streptokinase from equisimilis are all 97 to 98% homologous to each other. This fact can be confirmed by visiting the European BioInformatics Institute (EMBL-EBI) on-line at www.ebi.ac.uk. The EBI website contains on-line access to ClustalW, a widely used and trusted program for comparing nucleotide and amino acid sequences. Using the ClustalW program to evaluate the four streptokinases noted above reveals their extraordinary homology to one another.

In closing, Applicants respectfully submit that this rejection is improper because the phrase "streptokinase" is well-known, well-defined in the art, amply discussed in the specification, and well-understood by those of skill in the art. Thus, the rejection of Claims 1, 2, 4, 5, 7-13, and 15-21 under §112, first paragraph (written description) is untenable. Withdrawal of the rejection is now requested.

Rejection of Claims 2 and 7 Under §112, Second Paragraph:

Regarding Claim 2, this rejection is respectfully traversed because the tandem lambda PR/PL promoter (*i.e.*, "λpR-λpL" is Claim 2) is a known promoter, isolated from bacteriophage. This promoter can be purchased commercially, *e.g.*, ATCC 37830, see Exhibit D. As noted in Exhibit D, ATCC 37830 is a plasmid known as pCE30. The plasmid contains the lambda PR/PL promoter. In short, the term "λpR-λpL" is well understood to a person of ordinary skill in the art to designate a commercially available

promoter that will function in *E. coli*. Withdrawal of the rejection is respectfully requested.

Regarding Claim 7, Applicants submit that this rejection has been overcome by appropriate amendment to the claims, in accordance with the Examiner's recommendation. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 1 and 3 Under §102(b) in View of Pang (U.S. Patent No. 5,011,686):

This rejection is respectfully traversed because the applied reference fails entirely to mention a DNA expression construct that drives the transgenic production of **streptokinase** in the form of an inclusion body, either explicitly or inherently.

The Office's citation to Example 2 of the Pang patent is without basis. Streptokinase and urokinase are, **by definition**, different enzymes. Example 2 of the Pang patent does not describe, explicitly or inherently, the expression of streptokinase as an inclusion body. Further still, Example 2 **does not** describe the expression of urokinase per se, as an inclusion body. Rather, example 2 of the Pang patent describes the expression of a fusion protein as an inclusion body. The urokinase portion of the fusion protein has nothing whatsoever to do with the expression of the fusion protein as an inclusion body. If that were the case, naturally expressed urokinase would be expressed as an inclusion body, which it isn't. The inclusion body is driven by the tandem FB-FB portion of the Example 2 construct.

On this point, note that the plasmid pBr322 mentioned by the Office **does not** contain the tandem FB-FB fusion portion of the construct described in Example 2, nor a complete single FB fragment. Thus, the Office's assertion that this construct will drive the expression of streptokinase as an inclusion body is without merit. The Office is simply disregarding the contrary text of Example 3. In particular, note the passage at column 11, lines 3-7, of the Pang patent:

> The effect of restricting PI13 with MluI is to cut the DNA of both tandem FB encoding sequences at the unique MluI site, producing a fragment consisting of trailing nucleotides of the first FB and leading nucleotides of the second FB.

In short, it is the tandem FB-FB portion of Pang's Example 2 construct that drives expression of the urokinase as an inclusion body. Example 3 of Pang described a construct that **does not** include the tandem FB-FB portion. Therefore, the construct described in Pang's Example 3 **will not** express streptokinase as an inclusion body.

Thus, the Office's position that this patent inherently describes the expression of streptokinase as an inclusion body is incorrect. The doctrine of inherency excludes "possibilities." For the Office to make out a *prima facie* case of inherency, the inherent result must be an inescapable, inexorable result of the data presented in the applied reference. In the present instance, it is beyond argument that it is the FB-FB portion of Pang's construct that results in expression of a fusion protein. Insofar as Pang's construct described in Example 3 lacks this portion of the construct (as described in Example 2), there simply is no possibility whatsoever that Pang's Example 3 construct will drive the expression of streptokinase as a fusion protein.

Thus, the rejection of Claims 1 and 3 under §102(b) in view of Pang is untenable. Withdrawal of the same is respectfully requested.

Rejection of Claims 1-5, 8-9, and 11 Under §102(b) in View of Pupo et al. (1999) *Biotechnology Letters* 21:1119-1123:

This rejection is respectfully traversed because the Pupo et al. patent does not describe expressing streptokinase as an inclusion body. The Office cites vector pACS-2, at page 1120, as being a plasmid that drives the expression of streptokinase as an inclusion body. But this is not the case, as is made clear by Pupo et al. themselves. At page 1120, left-hand column, first full paragraph, the authors explicitly state that the streptokinase gene described therein was expressed "as a soluble protein" and **not** as an

inclusion body. See especially the explicit notation at page1122, left-hand column, wherein the authors state that the small amount of streptokinase that was found in the insoluble fraction of disrupted cells was caused by contamination of the insoluble fraction with the soluble fraction. Specifically, the authors found that "most of this protein [the streptokinase found in the insoluble fraction] could be removed by washing several times with PBS."

In short, the Pupo et al. paper focuses exclusively on producing streptokinase as a fully solublilized protein from the outset, and not as an inclusion body.

Applicants thus submit that the rejection of Claims 1-5, 8-9, and 11 under §102(b) in view of Pupo et al. is untenable. Withdrawal of the same is respectfully requested.

Rejection of Claims 4-5 and 8-11 Under §103(a) Over Pang in View of Marston (1984) Bio/Technology 2:800-804:

This rejection is respectfully traversed because the Pang reference fails entirely to mention a DNA expression construct that drives the transgenic production of **streptokinase** in the form of an inclusion body, either explicitly or inherently, and combining Pang with Marston does not cure the shortcomings of the Marston paper. In short, the combined references do not suggest or render obvious the invention now claimed because the teaching of the Pang reference has been mistakenly characterized.

As noted earlier, the Office's citation to Example 2 of the Pang patent is without basis. Streptokinase and urokinase are, **by definition**, different enzymes. Example 2 of the Pang patent does not describe, explicitly or inherently, the expression of streptokinase as an inclusion body. Further still, Example 2 **does not** describe the expression of urokinase per se, as an inclusion body. Rather, example 2 of the Pang patent describes the expression of a fusion protein as an inclusion body. The urokinase portion of the fusion protein has nothing whatsoever to do with the expression of the fusion protein as an inclusion body. If that were the case, naturally expressed urokinase would be

expressed as an inclusion body, which it isn't. The inclusion body is driven by the tandem FB-FB portion of the Example 2 construct.

On this point, note that the plasmid pBr322 mentioned by the Office **does not** contain the tandem FB-FB fusion portion of the construct described in Example 2, nor a complete single FB fragment. Thus, the Office's assertion that this construct will drive the expression of streptokinase as an inclusion body is without merit. The Office is simply disregarding the contrary text of Example 3. In particular, note the passage at column 11, lines 3-7, of the Pang patent:

> The effect of restricting PI13 with MluI is to cut the DNA of both tandem FB encoding sequences at the unique MluI site, producing a fragment consisting of trailing nucleotides of the first FB and leading nucleotides of the second FB.

In short, it is the tandem FB-FB portion of Pang's Example 2 construct that drives expression of the urokinase as an inclusion body. Example 3 of Pang described a construct that **does not** include the tandem FB-FB portion. Therefore, the construct described in Pang's Example 3 **will not** express streptokinase as an inclusion body.
Thus, the Office's position that this patent inherently describes the expression of streptokinase as an inclusion body is incorrect. The doctrine of inherency excludes "possibilities." For the Office to make out a *prima facie* case of inherency, the inherent result must be an inescapable, inexorable result of the data presented in the applied reference. In the present instance, it is beyond argument that it is the FB-FB portion of Pang's construct that results in expression of a fusion protein. Insofar as Pang's construct described in Example 3 lacks this portion of the construct (as described in Example 2), there simply is no possibility whatsoever that Pang's Example 3 construct will drive the expression of streptokinase as a fusion protein.

By the Office's own characterization, the Marston paper is wholly silent with regarding to streptokinase. The Marston paper is limited to a discussion of solubilizing prochymosin. Thus, the **combination** of the Pang and Marston does not describe or

suggest solubilizing a streptokinase-containing inclusion body because the primary reference (Pang) **does not** teach or suggest a construct that drives the production of a streptokinase-containing inclusion body and the secondary reference (Marston) is totally silent with regard to streptokinases entirely.

Thus, the rejection of Claims 4, 5, and 8-11 and 3 under §103(a) over Pang in view of Marston is improper. Withdrawal of the same is respectfully requested.

Applicants submit that the application is now in condition for allowance. Early notification of such action is earnestly requested.

Respectfully submitted,

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Report from the introduction to *Enzyme Nomenclature*

Historical Introduction

The sixth complete edition of *Enzyme Nomenclature*, was published under the auspices of the International Union of Biochemistry and Molecular Biology (formerly the International Union of Biochemistry). A brief history of the development of a standard terminology for enzymes is given in this chapter. By the late 1950's it had become evident that the nomenclature of enzymology, in the absence of any guiding authority, in a period when the number of known enzymes was increasing rapidly, was getting out of hand. The naming of enzymes by individual workers had proved far from satisfactory in practice. In many cases the same enzymes became known by several different names, while conversely the same name was sometimes given to different enzymes. Many of the names conveyed little or no idea of the nature of the reactions catalysed, and similar names were sometimes given to enzymes of quite different types. To meet this situation, various attempts to bring order into the general nomenclature of enzymes, or into that of particular groups of enzymes, were made by individuals or small groups of specialists, but none of the resulting nomenclatures met with general approval.

In view of this state of affairs, the General Assembly of the International Union of Biochemistry (IUB) decided, during the third International Congress of Biochemistry in Brussels in August, 1955, to set up an International Commission on Enzymes. This step was taken in consultation with the International Union of Pure and Applied Chemistry (IUPAC).

The International Commission on Enzymes was established in 1956 by the President of the International Union of Biochemistry, Professor M. Florkin, with the advice of an *ad hoc* Committee. The following members were appointed by the Bureau of the International Union of Biochemistry:

> A.E. Braunstein, U.S.S.R.; S.P. Colowick, U.S.A.; P.A.E. Desnuelle, France; M. Dixon, U.K. (*President*); V.A. Engelhardt, U.S.S.R.; E.F. Gale, U.K.; O. Hoffmann-Ostenhof, Austria; A.L. Lehninger, U.S.A.; K. Linderstrom-Lang, Denmark; F. Lynen, Germany.
>
> *Corresponding Members*: F. Egami, Japan; L.F. Leloir, Argentina.
>
> In 1959, on the death of K. Linderstrom-Lang, E.C. Webb (United Kingdom, later Australia) joined the Commission.

Of these members, O. Hoffmann-Ostenhof had already published a review on the problem (*Advances in Enzymology* (1953) **14**, 219-260) with considerable progress towards a classification, and M. Dixon and E.C. Webb had drafted a list for their book, *Enzymes*, which was to appear in 1958. The Commission brought together these efforts to list enzymes systematically.

The terms of reference of the Enzyme Commission, as laid down by the *ad hoc* Committee, were as follows:

> *'To consider the classification and nomenclature of enzymes and coenzymes, their units of activity and standard methods of assay, together with the symbols used in the description of enzyme kinetics.'*

The Enzyme Commission faced many difficulties arising from the uncontrolled naming of the rapidly increasing number of known enzymes. Some of the names in use were definitely misleading; others conveyed little or nothing about the nature of the reaction catalysed, as for example, *diaphorase, Zwischenferment, catalase*. Enzymes catalysing essentially similar reactions had sometimes names implying that they belong to different groups, while some enzymes of different types had been placed in the same group, for example, the *pyrophosphorylases* had included both glycosyltransferases and phosphotransferases. In some cases a name which had been well established for many years with a definite meaning, such as the term *synthetase*, had been later employed with different meanings, thus causing confusion.

One of the main tasks given to the Commission was therefore to see how the nomenclature of enzymes could best be brought into a satisfactory state, and whether a code of systematic rules could be devised that would serve as a guide for the consistent naming of new enzymes in the future. At the same time, the Commission realized the difficulties that would be caused by a large number of changes of well-known enzyme names, and the desirability of retaining the existing names wherever there was no good reason for making an alteration. Nevertheless, the overriding consideration was to reduce the confusion and prevent further confusion from arising. Its task could not have been accomplished without causing some inconvenience, for this was the inevitable result of having allowed the problem to drift for a considerable time.

Throughout its work, the Enzyme Commission was in close touch with the Biological Chemistry Nomenclature Commission of IUPAC. In addition, it considered many comments and suggestions from various experts in the field; 52 formal documents were circulated and discussed in several meetings. Finally, the Commission prepared a report, which was presented to the General Assembly of the International Union of Biochemistry at their meeting in Moscow in 1961, and was adopted for publication by Pergamon Press of Oxford. The nomenclature set out in that report has been widely used in scientific journals, textbooks, *etc.* since 1961.

Subsequently, the Council of IUB dissolved the Commission on Enzymes and set up a Standing Committee on Enzymes consisting of S.P. Colowick, O. Hoffmann-Ostenhof, A.L. Lehninger and E.C. Webb (*Secretary*). This Standing Committee discussed the comments and criticisms received on the published report of the Enzyme Commission and prepared in 1964 a second version, the *Recommendations (1964) of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes.* This edition was published by Elsevier Publishing Company of Amsterdam; it also became Volume 13 of *Comprehensive Biochemistry* (Florkin, M. & Stotz, E.H., ed.), published by Elsevier.

The function of the Standing Committee on Enzymes was then taken over by the IUPAC/IUB Commission on Biochemical Nomenclature (CBN). This Commission was originally set up to deal with the nomenclature of various compounds of biochemical interest. At a meeting in September, 1969, it was decided that the *Recommendations on Enzyme Nomenclature* should be revised, mainly to include the many enzymes discovered in recent years, and an Expert Committee on Enzymes was formed, consisting of A.E. Braunstein, J.S. Fruton, O. Hoffmann-Ostenhof, B.L. Horecker, W.B. Jakoby, P. Karlson, B. Keil, E.C. Slater, E.C. Webb (*convener*) and W.J. Whelan. With the help of a number of expert subcommittees, and comments and suggestions solicited from authors and editors, a completely revised version of *Enzyme Nomenclature* was prepared and published by Elsevier Publishing Company as *Recommendations (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry.* Once again it was also published as Volume 13 of *Comprehensive Biochemistry.*

After the publication of the third version of the complete report and enzyme list, the Commission on

Biochemical Nomenclature decided that it would be appropriate to publish from time to time a Supplement to the Enzyme List, containing new entries, deletions and corrections. The first supplement was prepared during 1974-5 and published in *Biochimica et Biophysica Acta* (1976) **429**, 1-45.

In 1977 the Nomenclature Committee of IUB (NC-IUB) was set up and responsibility for enzyme nomenclature passed to it. In this task it has worked closely with the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). The 1978 recommendations, published by Academic Press in 1979, were prepared with considerable help from the Division of Computor Research and Technology of the U.S. National Institutes of Helth, under the direction of Richard J. Feldmann.

Following the publication of the 1978 edition, the work of updating the enzyme list continued steadily, and NC-IUB published four supplements in the *European Journal of Biochemistry*: (1980) **104**, 1-4; (1981) **116**, 423-435; (1982) **125**, 1-13; (1983) **131**, 461-472. It was agreed in 1982 that the number of changes to existing entries, together with a substantial number of new entries, justified a completely new edition and this was published by Academic Press towards the end of 1984.

Since 1984 three further supplements have been published in the *European Journal of Biochemistry*: ((1986) **157**, 1-26; (1989) **179**, 489-533; (1990) **187**, 263-281). The additional entries to the enzyme list in these supplements, together with improvements to older entries proposed by individual authors or small groups of workers, were incorporated into a draft of the present volume by Edwin Webb. The final version was approved at a meeting of NC-IUB in May, 1991.

The Enzyme List in the sixth edition contains 3540 entries, of which 178 record enzymes which are now transferred elsewhere in the list, and 166 have been deleted completely. Thus the number of enzymes actually listed is 3196, an increase of 29% on the 1984 edition.

The size of the list has increased steadily since the publication of the Report of the Enzyme Commission, as shown in the following figures for 'live' entries:

Report of the Enzyme Commision (1961)	712
Enzyme Nomenclature (1964)	875
Enzyme Nomenclature (1972)	1770
Enzyme Nomenclature (1978)	2122
Enzyme Nomenclature (1984)	2477
Enzyme Nomenclature (1992)	3196

Many people have contributed to this steady growth. Particular mention will be made of a few. Otto Hoffmann-Ostenhof was Secretary of the original Enzyme Commission, and then Chairman of the Commission on Biochemical Nomenclature from 1965-76, and was largely responsible for continuity in listing over that time. Alexander E. Braunstein had a similarly long association with the work. Malcolm Dixon was President of the Enzyme Commission from 1956 to 1961, and he and Edwin Webb gave great impetus to listing by the publication of their book. Edwin Webb was secretary of the IUB Standing Committee on Enzymes (1961-68). At the University of Queensland, at Macquarie University and now in an honorary position at the James Cook University of North Queensland he has overseen entries into the list for nearly 30 years. Over this time he has had the able assistance of Miriam Webb (née Armstrong). NC-IUB is deeply grateful to him for the preparation of this edition for publication, as well as for all the work leading up to it.

In recent years Kurt Loening has arranged the compilation by John Wittorf of an annual list of enzyme names from *Chemical Abstracts* which do not appear to be listed in *Enzyme Nomenclature*. These lists

have proved to be a valuable base for further searches of the literature.

Not all sections of the enzyme list have received the same attention at each revision. For the present edition, subclass EC 3.4, the peptidases, has undergone the most thorough revision, based on the recommendations of an expert panel appointed by NC-IUB in 1989. The panel, consisting of A.J. Barrett (convener), J.S. Bond, F. Fiedler, J.K. McDonald, L. Polgár, S. Wilk and J.F. Woessner, Jr, revised the preamble to subclass 3.4, and recommended the deletion of 51 of the previous entries, the transfer of 50 enzymes to other numbers, and the inclusion of 56 entirely new entries.

It is the intention of NC-IUBMB to commission similar 'in depth' revision of sections of the enzyme list from time to time. A number of expert panels are being set up now to review some sections before the next edition of *Enzyme Nomenclature* is due. These include transaminases (EC 2.6.1), kinases (EC 2.7.1), nucleases (EC 3.1.11-31), lipases, ribozymes, and protein kinases and protein phosphatases. Biochemists who feel able to contribute in these areas are invited to write to Professor K.F. Tipton, Department of Biochemistry, Trinity College, Dublin 2, Republic of Ireland, who will be co-ordinating the activities of NC-IUB in the field of enzyme nomenclature.

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IUBMB Enzyme Nomenclature

EC 3.4.21.7

Recommended Name: plasmin

Reaction: Preferential cleavage: Lys+ > Arg+; higher selectivity than trypsin. Converts fibrin into soluble products

Other names: fibrinase; fibrinolysin; actase; serum tryptase; thrombolysin

Comments: Formed from plasminogen by proteolysis which results in multiple forms of the active plasmin. In peptidase family S1 (trypsin family). Formerly EC 3.4.4.14

Links to other databases: BRENDA, EXPASY, MEROPS, PDB, CAS registry number: 9001-90-5

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2. Castellino, F.J. and Powell, J.R. Human plasminogen. *Methods Enzymol.* 80 (1981) 365-378. [Medline UI: 82194866]

3. Robbins, K.C., Summaria, L. and Wohl, R.C. Human plasmin. *Methods Enzymol.* 80 (1981) 379-387. [Medline UI: 82194867]

[EC 3.4.21.7 created 1961 as EC 3.4.4.14, transferred 1972 to EC 3.4.21.7]

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EXHIBIT B

PDBsum Enzymes E.C.3.4.21.7

E.C.3.-.-.- Hydrolases.

E.C.3.4.-.- Acting on peptide bonds (peptide hydrolases).

E.C.3.4.21.- Serine endopeptidases.

E.C.3.4.21.7 Plasmin.

BROOKHAVEN PROTEIN DATA BANK (The PDB)

Reaction: *Preferential cleavage: Lys-|-Xaa > Arg-|-Xaa; higher selectivity than trypsin. Converts fibrin into soluble products.*
Other name(s): *Fibrinase. Fibrinolysin.*
Comments: *Formed from plasminogen by proteolysis which results in multiple forms of the active plasmin. Belongs to peptidase family S1.*

Links to other enzyme databases: ExPaSy KEGG WIT BRENDA

There are **18** PDB entries in enzyme class E.C.3.4.21.7

- 1b2i (PDB *Chain A* **2.40.20.10**)
  Structure: *1. Plasminogen. Fragment: kringle 2 domain.*
  Source: *1. Homo sapiens. Human. Expression_system_vector_type: plasmid.*
- 1bml (PDB *Chain A* **2.40.10.10**, *Chain B* **2.40.10.10**, *Chain C* **3.10.20.150**, **3.10.20.180**, *Chain D* **3.10.20.150**, **3.10.20.180**)
  Structure: *1. Plasmin. Fragment: catalytic domain. 2. Streptokinase.*
  Source: *1. Homo sapiens. Human. Expression_system_common: bacteria. Expression_system_vector: pet11. 2. Streptococcus equisimilis. Bacteria*
- 1bui (PDB *Chain A* **2.40.10.10**, *Chain B* **2.40.10.10**, *Chain C* **3.10.20.130**)
  Structure: *1. Plasmin. Fragment: catalytic domain. 2. Staphylokinase sak-c-phi-c. 3. Inhibitor.*
  Source: *1. Homo sapiens. Human. 2. Bacteriophage 42d. 3.*
- 1cea (PDB *Chain A* **2.40.20.10**, *Chain B* **2.40.20.10**)
  Structure: *1. Plasminogen. Fragment: kringle 1. Synonym: k1pg.*
  Source: *1. Homo sapiens. Human.*
- 1ceb (PDB *Chain A* **2.40.20.10**, *Chain B* **2.40.20.10**)
  Structure: *1. Plasminogen. Fragment: kringle 1. Synonym: k1pg.*
  Source: *1. Homo sapiens. Human.*
- 1ddj (PDB *Chain A* **2.40.10.10**, *Chain B* **2.40.10.10**, *Chain C* **2.40.10.10**, *Chain D* **2.40.10.10**)
  Structure: *1. Plasminogen. Fragment: catalytic domain.*
  Source: *1. Homo sapiens. Human. Tissue: blood. Expression_system_common: bacteria. Expression_system_vector_type: plasmid.*
- 1hpj (PDB **2.40.20.10**)
  Structure: *1. Plasminogen. Fragment: kringle 1 domain.*
  Source: *1. Homo sapiens. Human. Tissue: blood plasma*

EXHIBIT C

- **1hpk** (2.40.20.10)
  Structure: *1. Plasminogen. Fragment: kringle 1 domain.*
  Source: *1. Homo sapiens. Human. Tissue: blood plasma*
- **1i5k** (*Chain A* **2.40.20.10**, *Chain B* **2.40.20.10**)
  Structure: *1. Plasminogen. Fragment: modified recombinant kringle-2 domain. 2. M protein. Fragment: vek-30 (30 residue internal peptide).*
  Source: *1. Pichia pastoris. Yeast. Expression_system_common: yeast. 2. Phase peptide synthesis*
- **1ki0**
  Structure: *1. Angiostatin.*
  Source: *1. Homo sapiens. Human. Expression_system_common: fungus*
- **1krn** (2.40.20.10)
  Structure: *1. Plasminogen. Fragment: kringle 4 domain.*
  Source: *1. Homo sapiens. Human. Tissue: blood*
- **1l4d**
  Structure: *1. Plasminogen. Fragment: catalytic domain, residues 543-791. 2. Streptokinase. Fragment: alpha domain, residues 14-147.*
  Source: *1. Homo sapiens. Human. Expression_system_common: bacteria. Expression_system_vector_type: plasmid. 2. Streptococcus dysgalactiae subsp. Equisimilis. Bacteria. Expression_system_common: bacteria. Expression_system_vector_type: plasmid.*
- **1l4z**
  Structure: *1. Plasminogen. Fragment: catalytic domain, residues 544-791. 2. Streptokinase. Fragment: n terminal alpha domain, residues 0-147.*
  Source: *1. Homo sapiens. Human. Expression_system_common: bacteria. Expression_system_vector_type: plasmid. Expression_system_vector: pet11. 2. Streptococcus dysgalactiae subsp. Equisimilis. Bacteria. Expression_system_common: bacteria. Expression_system_vector_type: plasmid. Expression_system_vector: pet11*
- **1pk4** (2.40.20.10)
  Structure: *Plasminogen kringle 4*
  Source: *Human (homo sapiens)*
- **1pkr** (2.40.20.10)
  Structure: *Plasminogen (kringle 1 domain)*
  Source: *Human (homo sapiens) recombinant form expressed In (escherichia coli)*
- **1pmk** (*Chain A* **2.40.20.10**, *Chain B* **2.40.20.10**)
  Structure: *Plasminogen kringle 4*
  Source: *Human (homo sapiens)*
- **2pk4** (2.40.20.10)
  Structure: *Human plasminogen kringle 4 complex with epsilon- Aminocaproic acid*
  Source: *Human (homo sapiens)*
- **5hpg** (*Chain A* **2.40.20.10**, *Chain B* **2.40.20.10**)
  Structure: *1. Plasminogen. Fragment: kringle 5.*
  Source: *1. Homo sapiens. Human.*

pCE30

- **Vector IG Sequence Link :**
- **General :** plasmid ds-DNA 4001 BP
- **Functions :** (cloning)
- **Selection :** ()
- **Copy Number :**
- **Hosts :** (E.coli AN1459)(E.coli)
- **Suppliers :** (ATCC)
- **Misc.Comments :** Constructed by inserting a 1349 bp fragment from pMY17-3 containing the cI857 gene and tandem lambda PR and PL promoters into pUC9. The promoters are immediately upstream of the cloning sites. The sequence surrounding the cloning sites has been used to design primers for direct sequencing, including the M13 universal primer. Because cI857 is expressed by the plasmid itself, from its natural promoter PM, the vector may be used in virtually any E.coli strain. Expression vector containing primer sites useful for sequencing and encoding cI857. (ATCC staff) Restriction digests of the clone give the following sizes (kb): BamHI--4.0; BamHI/EcoRI--4.0; BglI--2.9, 1.1. (ATCC staff) Medium is 1227 LB plus ampicillin. NCBI gi: 208976
- **Parents :** ()
- **Siblings :** ()
- **Descendents :** ()

- **NCBI ENTREZ Link :**

Return to Vector Homepage

EXHIBIT D

Return to this vector's summary.

```
ID   PCE30      preliminary; circular DNA; SYN; 4001 BP.
XX
AC   M36426; ATCC37830;
XX
DT   01-JUL-1993 (Rel. 7, Created)
DT   01-JUL-1995 (Rel. 12, Last updated, Version 1)
XX
DE   E. coli plasmid vector pCE30 - incomplete, promoter.
XX
KW   cloning vector.
XX
OS   Cloning vector
OC   Artificial sequences; Cloning vehicles.
XX
RN   [1]
RC   pCE30 from pUC9 & pMY17-3, lambda cI857 gene/pR/pL
RC   pCE33 from pCE30 & pMOB45
RC   pND201 from pCE30 & linker
RC   pPT150 from pCE30 & pBR322
RC   pMA200U from pCE30 & pTZ18U
RA   Elvin C.M., Thompson P.R., Argall M.E., Hendry P., Stamford N.P.,
RA   Lilley P.E., Dixon N.E.;
RT   "Modified bacteriophage lambda promoter vectors for overproduction
RT   of proteins in Escherichia coli";
RL   Gene 87:123-126(1990).
XX
CC   Constructed by inserting a 1349 bp fragment from pMY17-3 containing
CC   the cI857 gene and tandem lambda PR and PL promoters into pUC9. The
CC   promoters are immediately upstream of the cloning sites.
CC   The sequence surrounding the cloning sites has been used to design
CC   primers for direct sequencing, including the M13 universal primer.
CC   Because cI857 is expressed by the plasmid itself, from its natural
CC   promoter PM, the vector may be used in virtually any E.coli strain.
CC   Expression vector containing primer sites useful for sequencing and
CC   encoding cI857. (ATCC staff)
CC   Restriction digests of the clone give the following sizes (kb):
CC   BamHI--4.0; BamHI/EcoRI--4.0; BglI--2.9, 1.1. (ATCC staff)
CC   Medium is 1227 LB plus ampicillin.
CC   NCBI gi: 208976
CC   NM (pCE30)
CC   CM (no)
CC   NA (ds-DNA)
CC   TP (circular)
CC   ST ()
CC   TY (plasmid)
CC   SP (ATCC)
CC   HO (E.coli AN1459)(E.coli)
CC   CP ()
CC   FN (cloning)
CC   SE ()
CC   PA ()
CC   BR ()
CC   OF ()
CC   OR ()
XX
FH   Key             Location/Qualifiers
```

```
FH
FT   misc_feature    0..0
FT                   /note="1. pBR322 4361bp
FT                   2. lambda dv021, O gene N-terminal region
FT                   -> pOA-4
FT                   1. pBR322
FT                   2. lambda dv021
FT                   -> pBlambda4
FT                   1. pBR322
FT                   2. lambda, cI857ts gene/pL promoter/pR promoter
FT                   -> pMY12-6
FT                   1. pMY12-6
FT                   2. pOA-4
FT                   3. pBlambda4
FT                   -> pMY17-3
FT                   1. pUC9 PstI 2665bp 246..246
FT                   2. pMY17-3 PstI-PstI 1349bp, lambda cI857 gene/pR/pL
FT                   -> pCE30 4001bp"
FT   misc_binding    0..0
FT                   /note="MCS unique BamHI-SmaI-EcoRI"
FT   rep_origin      0..0
FT                   /note="ORI E. coli pMB1 (ColE1 and pBR322)"
FT   promoter        0..0
FT                   /note="PRO bacteriophage lambda PL"
FT   promoter        0..0
FT                   /note="PRO bacteriophage lambda PR"
FT   CDS             0..0
FT                   /note="REP bacteriophage lambda cI857 repressor gene"
FT   CDS             0..0
FT                   /note="ANT E. coli beta-lactamase gene (bla)
FT                   ampicillin resistance gene (apr/amp)"
XX
SQ   Sequence 143 BP; 37 A; 35 C; 40 G; 31 T; 0 other;
     agggcagcat tcaaagcaga aggctttggg gtgtgtgata cgaaacgaag cattgggatc
     cccgggaatt cactggccgt cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc
     caacttaatc gccttgcagc aca
//
```